

Further Observations on the Cæcal Parasites of Fowls, with Some Reference to the Rectal Fauna of other Vertebrates.

PART I.

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With Plates 10-14 and 4 Text-figures.

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1. GENERAL INTRODUCTION.

DURING the last two years we have been engaged in some work on the cæcal parasites of the fowl, and although we do not feel that our work is complete, we have decided to publish some of the results we have obtained.

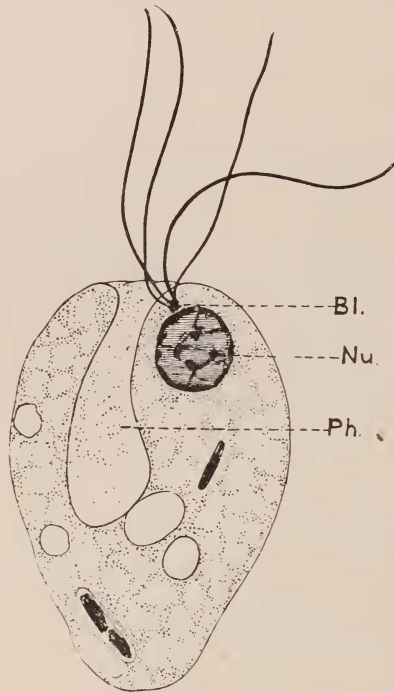
In recent years there seems to us to have been a tendency to regard the life-cycle of parasitic flagellates as a subject on

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which the most hazardous speculations can be put forward, and these, unfortunately, crystallise only too soon into the well-known protozoon life-cycles of the zoological text-book.¹

Although we do not feel justified in putting forward a complete life-cycle in the case of any of the forms described

TEXT-FIG. 1.



Chilomastix gallinarum, showing the four free flagella, blepharoplast, well-developed pharynx, and nucleus.

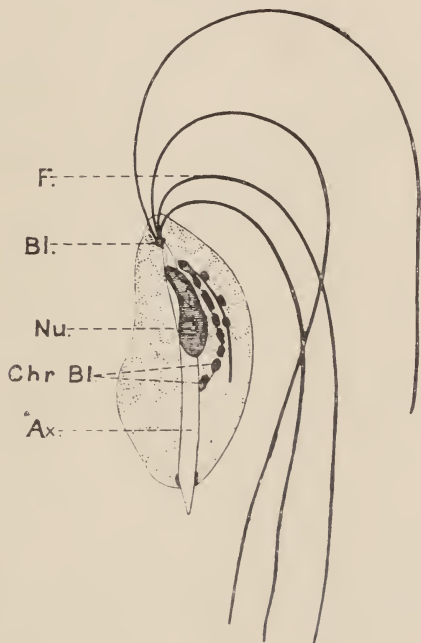
Abbreviations.—*Ax.* Axostyle. *Bl.* Blepharoplast. *b.g.* Basal granules. *Chr. B.* Chromatic blocks. *Chr. L.* Chromatic line. *Cyt.* Cytostome. *F.* Free flagellum. *Ka.* Karyosome. *M.F.* Membrane flagellum. *Nu.* Nucleus. *Ph.* Pharynx.

¹ In this the first part of our paper we only deal with the morphology of the flagellate parasites we have found. In the second part we give an account of the other animal parasites and of the results obtained from new infections.

below, we feel that we are justified in publishing the results obtained for the following reasons :

(1) Our preparations were always made from the wall of the cavity containing the parasite, and not simply from the content of the cavity. Owing, we believe, largely to this fact, we have not only obtained a far more complete series of

TEXT-FIG. 2.



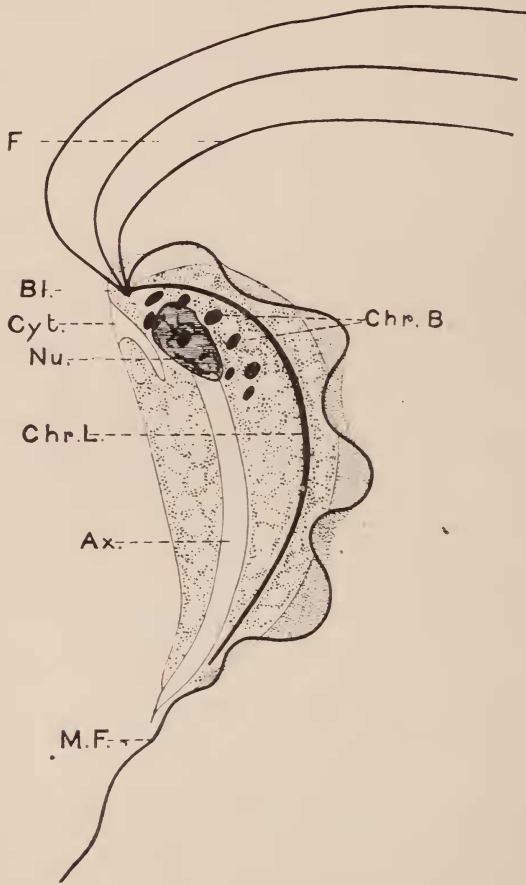
Trichomonas gallinarum, showing four free flagella, membrane flagellum, blepharoplast, cytostome, nucleus, chromatic blocks, and chromatic line.

division of the active forms that seems to have fallen to the lot of the earlier workers on similar forms, but we have also got an interesting series of new stages which must, we are convinced, play an important part in the final construction of the life-cycle of these forms.

(2) Owing to the ease with which it is possible to obtain newly hatched chicks and keep them free from all animal

parasites, we have obtained some really trustworthy data as to the course run by new infections of these forms.

TEXT-FIG. 3.

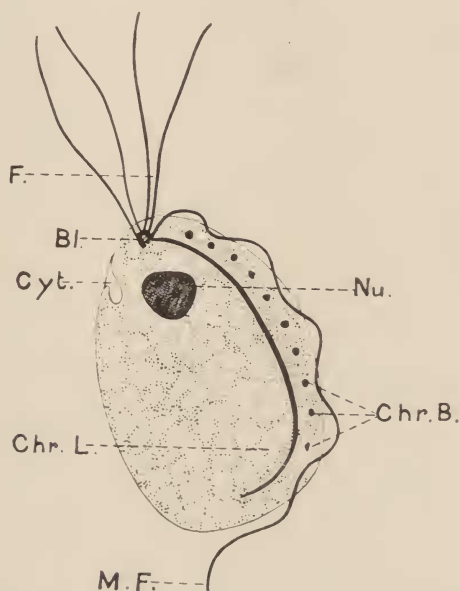


Trichomonas Eberthi, showing three free flagella, membrane flagellum, blepharoplast, cytostome, nucleus, chromatic blocks, chromatic line, and axostyle.

Until, of course, the whole life cycles of these animals are known, the breaking up of the complex series of forms inhabiting the cæca of fowls into good species is a matter of great difficulty. However, it must be remembered that the

same difficulty has to be met in the case of a vast majority of free-living flagellates, and, probably, in that of all the free-living Amœbæ; and partly by means of pure infections obtained in our series of infected chicks, and partly by comparison with similar parasites in other vertebrates, we have obtained evidence which we believe justifies us in dividing

TEXT-FIG. 4.



Trichomastix gallinarum, showing four free flagella, blepharoplast, cytostome, nucleus, chromatic blocks, chromatic line, and axostyle.

the animal parasites found in the cæca of fowls into the following species. [The evidence on which this division is based will, of course, be found in the special part of the paper.]

- (1) *Chilomastix gallinarum* (sp. nov.).
- (2) *Trichomonas gallinarum* (sp. nov.).
- (3) *Trichomonas Eberthi* (sp. nov.).
- (4) *Trichomastix gallinarum* (sp. nov.).

The chief points of resemblance and difference between the

typical active forms of these flagellates are shown in the following table, and the nomenclature that we have adopted for the various parts can be seen in the text-figures.

The literature upon rectal protozoan parasites has in recent years attained such dimensions that it is impossible to give here a detailed historical account of the work done by other observers. Under these circumstances, we have decided to content ourselves with discussing in our final summary the more obvious differences between our results and those of earlier workers on other forms. On the other hand, the literature dealing specifically with the protozoan fauna of the alimentary tract of the fowl is very limited, and as we have already discussed it fairly fully in our preliminary account of these forms, we need here only refer to one or two essential points.

Eberth, in 1862, first described these flagellate parasites from the cæcum of the fowl as "Ein kleines Infusorium," though he did not give them any name.

Stein, in 1878, first put forward the view that these parasites were Trichomonads in which the anterior flagella had been overlooked, and Leuckart, in the second edition of his book, 'Die Parasiten des Menschen,' arrived at the same conclusion. (In his first edition he placed these forms in a new genus—*Saenolophus*.)

This view of the Trichomonad affinities of these forms was later disregarded by Saville Kent in his monograph of the Infusoria (1881), where the name of *Trypanosoma eberthi* was given to these flagellates, and in this view he was followed by Bütschli (1889). Most of the later workers on parasitic flagellates—Dofflein, Laveran, Mesnil—have regarded these parasites as Trichomonads, but have not furnished any evidence in favour of this view. Lühe, in a short note in Mense's 'Handbuch der Tropenkrankheiten,' has changed the name from *Trypanosoma Eberthi* to *Spirochæta Eberthi*, probably on account of Eberth's original figures, in which the only part of the animal that is clearly shown is an undulating membrane. It is evident, we think,

Table of the Main Features characteristic of the Species dealt with.

Name	Cell body.	Free flagella	Membrane flagellum.	Chromatic blocks.	Chromatic line.	Nucleus.	Axostyle.
<i>Chlomastix gallinarum</i>	Rounded, very well-developed pharynx. Vacuolar cytoplasm	4	—	—	—	Round and vacuolar. The chromatin in part condensed on the nuclear membrane, in part on central masses	—
<i>Trichomonas gallinarum</i>	Rounded. Cytostome small	4	Present	A single line of small blocks parallel to the base of the membrane	Present; runs down the whole length of the body parallel to the base of the membrane	Rounded. Chromatin in small granules throughout the nucleus	—
<i>Trichomonas Eberthi</i>	Carrot-shaped body. Cytostome small	3 long flagella	Present membrane very deep	Large blocks in a double row near the anterior end over the nucleus	Present; runs down the body parallel to the base of the membrane	Elongated oval. Chromatin distributed between a coarse internal reticulus and the membrane	Well developed
<i>Trichomastix Gallinarum</i>	Carrot-shaped body.	4 long flagella	—	Large blocks running half way down one side of the animal's body from the anterior end	Short and inconspicuous; concealed by the blocks	Elongate oval. Chromatin very dense	Present

from the facts mentioned below, that all these names—*Saenolophus* (Stein), *Trypanosoma Eberthi* (Kent), and *Spirochæta Eberthi* (Lühe) must now lapse.

2. CÆCA OF FOWLS.

Before proceeding to the special part of the paper it will be necessary to give a short account of the cæca of the intestine of fowls, since it is this portion of the alimentary tract to which these parasites seem to be confined.

According to Newton ('Dictionary of Birds,' p. 18), "it is highly probable that originally all birds possessed cæca, and that according to the diet these were either further developed or reduced in size, or even lost ultimately."

In the case of the fowl the cæca are two blind sacs, rather variable in size, arising at the proximal end of the rectum. As a general rule the two cæca are found in a distended condition, and it would appear that almost the whole process of fæces formation in the fowl takes place in these organs. In the early stages of this process the cæca are filled with a light yellowish, rather fluid mass, which appears to be identical with the content of the intestine. In the later stages the content of the cæcum becomes gradually darker and more solid, acquiring at the same time the typical fæcal odour.

It may be well to point out that during early stages of fæces formation the rectum, i. e. the portion of the alimentary tract below the opening of the cæca, is always empty, and it is only in the later stages where the cæcal content has already acquired the characteristic fæcal appearance that anything is found in the rectum itself.

We had hoped that it might be possible to associate certain stages in the parasites with stages in this conversion of the cæcal content into the fæces. In this we have not been successful. On the other hand, there is a very distinct and characteristic change in the appearance of the bacterial flora during this process of fæces formation, to which we will return in a later part of the paper.

There is one interesting point in regard to the cæca of the newly hatched chick at a time before the yolk-sac is completely absorbed and before the animal has begun to take food. In these cases the cæca are periodically filled with a bright green fluid, which gives all the characteristic reactions for bile-pigment. It would seem to us, therefore, that the cæca must play an important part in the economy of the fowl, since even in these young chicks, in which the intestine is practically empty, and the only stuff passing down is presumably the bile, every trace of this is collected for some time in the cæca on its way to the exterior.

3. METHODS.

It is a common complaint amongst workers on rectal parasites that division forms are relatively rare: e. g. Dobell, in his paper on the "Intestinal Protozoa of Frogs and Toads," says regarding *Trichomastix batrachorum*, on p. 209, "stages in division are very difficult to find"; and again, as regards *Trichomonas batrachorum*, on p. 217, "I have not found so many division stages in *Trichomonas* as in *Trichomastix*, but the likeness between them is so great that I have little doubt that they correspond almost identically."

And, in fact, one of the most recent workers on these forms—Bensen, in his paper on "*Trichomonas intestinalis* und *vaginalis* des Menschen"—only refers to the possibility of division of the active flagellates in the following passage, p. 122: "Daneben können auf dem Amöboidstadium und im Anfang der Cysten bildung, nach früheren Beobachtern auch im Flagellatzustand, Vermehrungen durch gewöhnliche Zweiteilung stattfinden."

This is, we believe, largely due to the fact that the smears used by these workers were made from the rectal content, whereas, as far as can be found in the fowl and the fish which we have examined, the chief dwelling-place of these parasites is in the layer of mucus separating the gut content

from the wall, and, at any rate in the fowl, most of the division stages occur in the actual crypts of the wall itself.

The method we adopted in the examination of our fowls was to open up the cæcum, and after cutting away a portion of the cæcal wall to smear it on the surface of a clean coverslip. The smears were then plunged into a tube containing either strong Flemming or corrosive acetic.

Both fixatives gave excellent results. The Flemming smears were washed out, according to the method recommended by Overton, with a solution of a little hydrogen peroxide in 70 per cent. alcohol, while the corrosive smears were washed out with a weak iodine solution in 70 per cent. alcohol.

The most serviceable general stains were found to be dilute acid Mayer's hæmalum counter-stained by eosin, though in addition we made use of a large number of preparations with other stains, e. g. iron-hæmatoxylin, Giemsa, Twort, Delafield's hæmatoxylin, borax carmine, alum carmine, safranin, methyl green; and as counter-stains we also found orange G., Licht-grün and picric acid, and picric acid alone, valuable.

Sections were found only to be of value as an indication of the distribution of the parasites in the gut. As has already been stated, the great advantage which the fowl offered over all other animals for the study of these gut parasites seemed to us to lie not only in the great ease with which it was possible to obtain uninfected individuals from eggs hatched in incubators, but also in the fact that it was quite easy to keep the young chicks free from all animal infection for weeks. As far as we are aware none of the other animals which has hitherto been used for work on similar parasites, viz. the frog, lizard, mouse, man, is so easily handled; since, in the first place, with the fowl it is quite easy with suitable incubators to get a practically unlimited supply of uninfected material almost throughout the year; secondly, the animals can be easily kept in a healthy condition on sterilised food; thirdly, the cæca in young chicks are just a convenient size to handle, and they are not too big to prevent the fixed

smears representing a very faithful picture of the actual state of infection. To the results obtained by this method we return in the second part of our paper.

4. CHILOMASTIX GALLINARUM.

As will be seen from the table of infected fowls in Part II of this paper, this clearly marked species is a relatively rare parasite of fowls, but in those individuals in which it occurs it is always abundant.

For example, in No. 5, which is one of the chicks obtained from Llanfoist, the cæcal smears are one mass of *Chilomastix gallinarum*, which was, in fact, the only flagellate present, although neither of the other chicks which were obtained at Llanfoist at the same time showed any trace of the parasite.

In addition to its well-marked morphological characteristics, *Chilomastix gallinarum* is sharply marked off from the other flagellates in the fowl by two features in its behaviour.

In the first place, the division stages which are so common in the other flagellates, are, even in fowls well infected with this form, relatively rare.

Secondly, the encystation stages, which in other flagellates are extraordinarily rare, are, in the case of *Chilomastix*, actually common.

Chilomastix gallinarum can readily be recognised in live smears by its relatively large size, its spherical form, and large number of ingested bacteria. It moves on the warm stage fairly rapidly, in a rotary fashion. The body of the active *Chilomastix* is roughly spherical, though sometimes it is prolonged at the aboral end into a pointed tail. The body measures, roughly, on an average 9μ by 7μ , though larger and smaller forms are to be met with (Pl. 10, figs. 1-4).

From the anterior end of the body there are four fairly large flagella arising from a mass of darkly staining basal granules—the blepharoplast complex. It seems probable that there are four granules in all, and that each flagellum

arises from a separate granule. Near the origin of the flagella is the opening of the well-developed pharynx, which is one of the most characteristic features of this genus, and, as will be shown later, serves as a control in the case of doubtful encysting forms.

The pharynx is roughly horn-shaped, the narrow portion of the horn running back to end in the cytoplasm. The inner side of the pharynx, i. e. the side towards which the nucleus is applied, projects into the lumen of the pharynx as a well-marked rib; the cytoplasm has rather a loosely vacuolar appearance, and in active specimens is crammed with large food vacuoles containing bacteria.

The nucleus in the active animal is very characteristic, both as regards its appearance and position. It consists of a large vacuole containing well-developed chromatic masses, but the greater portion of the chromatin is condensed upon the nucleus wall.

The nucleus lies near the anterior pole of the animal, in close proximity to the opening of the pharynx, so that in the majority of specimens it obscures the origin of the four flagella.

As we have remarked above, we have not been able to obtain nearly as complete a series of divisions in the case of *Chilomastix* as in that of the other flagellates. Still, as will be seen from the figures of Pl. 10, the main outlines of the process are fairly clear. Division in *Chilomastix* is clearly transverse to the longitudinal axis of the animal's body.

An early stage is seen in Pl. 10, fig. 5. Here the blepharoplast complex has already divided, and two basal granules, bearing two flagella, have passed to a position near the animal's posterior pole, where there is already an in-sinking, the first sign of the new pharynx. It seems probable that the new pharynx arises as a bud from the old one.

The nucleus has already divided, and it is interesting to note that each of the daughter-nuclei appears to contain the same number of chromatin masses.

In the still later stages of division (Pl. 10, fig. 6) the new

pharynx is well developed, but there is as yet no sign of the outgrowth of new flagella. It is possible that the pointed form (Pl. 10, fig. 2) represents an individual which has just undergone division.

Encystation.—The earliest stage of encystation that we have found is shown on Pl. 10, fig. 7. The form here figured still preserves the rounded shape characteristic of the active individual, but the flagella have disappeared, and the pharynx is still well developed.

In the next stage (Pl. 10, fig. 8) the animal has become much smaller, and at the same time more oval, and a delicate wall has been secreted from its periphery. The remains of the blepharoplast are no longer to be detected, but it has possibly been absorbed into the nucleus, which has now passed from its former anterior to a more central position.

In the next stage the cyst has practically assumed its definite shape. The nucleus has undergone a good deal of shrinkage, leading up to the solid, darkly staining mass seen in the ripe cyst (Pl. 10, fig. 10). The changes undergone in the pharynx during this process are rather difficult to understand. It seems probable that, at any rate in the majority of cases, the thickened lining of the cytostome becomes loosened, and springing out of its original curved shape, lies as a darkly staining line running down one side of the cyst. A darkly staining cap at the anterior pole of the ripe cyst is due to a local differentiation of the cyst-wall. The whole of this process of encystation takes place in the cæcum, and ripe cysts have been found in the fæces some time after deposition. No trace of a sexual process has yet been found in this cycle.

In connection with this form we should like to draw attention to what is apparently a closely allied species, which was found by one of us to be very common in the rectum of the Saithe, *Gadus virens*, at Millport. It agrees closely with *Chilomastix gallinarum*, except for the fact that the terminal point is far more developed. We figure this animal in the second part of the paper.

5. *TRICHOMONAS GALLINARUM*.

Trichomonas gallinarum, as will be seen from the table, is one of the commonest flagellate parasites of the fowl.

It is rather variable in size ($5.4-7 \mu$ long by $5-6 \mu$ wide). Usually the form is more or less spherical, but sometimes the longitudinal axis of the body is considerably elongated (cf. Pl. 11, figs. 11, 12).

At the anterior end four free flagella arise from a complex blepharoplast, and another flagellum, with a similar origin, passes down one side of the animal, attached to a fairly well-developed membrane, ending freely posteriorly.

Arising from the blepharoplast, and passing down under the membrane is a darkly staining line. This has been termed by Dobell in the case of *Trichomonas batrachorum* the "chromatic base" of the membrane, but we are inclined to regard this structure as homologous with the line found in *Trichomastix gallinarum*, and therefore would prefer to use the term "chromatic line." We return to the very important part played in division by this structure in a later part of the paper.

Lying parallel to the line and between it and the base of the membrane there are, in the vast majority of cases, a number of well-marked blocks, which stain readily with chromatic stains. Similar blocks have been found in some species of trypanosomes, *Trypanoplasma*, *Trichomonas*, *Trichomastix*, and *Trypanophis*.

Near the origin of the flagella there is the aperture of a short curved pharynx. This is not nearly so well marked in this form as in the species which we describe under the name of *Trichomonas Eberthi*, and can only be seen in favourable specimens.

The nucleus is typically a fairly large oval structure and seems to contain a large quantity of closely packed chromatin granules, but there is a form of nucleus, the significance of

which is not fully understood, in which nearly all the chromatin is condensed on a large central karyosome. This form has been hitherto especially met with in recent infections. A slender axostyle is sometimes found in this form.

Our series of divisions for this form is fairly complete, and we think that it shows clearly that in its early stages division may occur in any one of three planes. These divisions may be termed respectively longitudinal, transverse, and oblique. The determining factors between these three forms of division appear to be: (1) direction of division of the blepharoplast complex; and (2) direction of the outgrowth of the new chromatic line. In all three cases the first process consists in the separation of the basal granules forming the blepharoplast into two moieties, each of which retains two of the original free flagella. The two new blepharoplasts arising in this way are connected by a darkly staining chromatic band.¹ One of the new blepharoplasts retains its old position and its connection with the old chromatic line and the old membrane flagellum. The other new blepharoplast moves away in a direction which is either at right angles or parallel to the longitudinal axis of the animal. From this blepharoplast a new chromatic line quickly grows out, and it will be seen from Pl. 11, figs. 15-30 more clearly than from any lengthy description that the initial plane of division depends largely upon the direction of the outgrowth of the new chromatic line. If, as in the cases Pl. 11, figs. 15-17, the line connecting the new blepharoplasts is at right angles to the longitudinal axis of the animal, and the new line grows out parallel to, and in the same direction as the old line, the division may be fairly described as longitudinal. If the line connecting the new blepharoplasts is parallel to the longitudinal axis of the animal's body, and the new line grows out parallel to, but in the reverse sense to the old line, the initial stages of the division are transverse (Pl. 11, figs. 18-24). If, finally, the

¹ This band has been regarded by other workers on similar forms as the axostyle. We can find no evidence for this view in this form, but we return to this point in our final summary.

line connecting the blepharoplasts is transverse to the longitudinal axis of the animal, as in the first case, but the new line grows out at right angles to the old line, the initial stages of division may be described as oblique (Pl. 11, figs. 25-27).

As regards the details of the division process, there seems to be a complete harmony between all three methods, though at the same time there is clearly a certain amount of time variation in regard to the various details of this process even in specimens undergoing a similar type of division. The general behaviour of the blepharoplast complex and the chromatic line has already been noted above, but it is necessary here to point out that we are completely in the dark as to the manner in which the chromatic blocks get re-arranged in the course of division. There seems to be some reason to believe that in the early stages of division the chromatic blocks become scattered throughout the cytoplasm, but it is quite clear from Pl. 11, figs. 21 and 24, that in the later stages of this process they have again attained their characteristic position under the base of the membrane flagellum.

The new membrane flagellum seems to grow out rather rapidly from the new blepharoplast at some fairly early period in the division process. The behaviour both of the membrane flagellum and the chromatic blocks during division present very sharply marked differences from the behaviour of the similar structures in *Trichomonas Eberthi* during this process. The two new free flagella in each of the division products grow out as rather thick, black processes from the blepharoplast complex. The stage at which this grows seems to show a certain amount of variability (see Pl. 11, figs. 28 and 26). The band joining the divided blepharoplasts seems to act as a sort of guide to the division of the nucleus, since it is always parallel to an imaginary line joining the two new nuclei; in fact the relation between the band joining the blepharoplasts and the dividing nuclei seems to be a very intimate one, and to have certain points of resemblance to some of the phenomena seen in the mitosis of a typical

metazoan nucleus. On the other hand, we wish to emphasise the fact that there is no evidence for regarding the process of nuclear division in this form as anything but an amitosis. It would seem that in the later stages of division separation of the cytoplasm may proceed more rapidly either at the anterior or the posterior pole, and this would explain such appearances as are shown in Pl. 11, figs. 29 and 30, since here we have what we must regard as two late stages of a longitudinal division, which, however, are clearly not successive. In Pl. 11, fig. 29, the in-cutting is proceeding more rapidly at the posterior pole, with a result that the two individuals will, at a slightly later stage, lie in a head-to-tail position, whereas in Pl. 11, fig. 30, the in-cutting is proceeding more rapidly from the anterior end, and the two new individuals will lie tail to tail.

The last two figures of *Trichomonas gallinarum* (Pl. 12, figs. 31, 32) are given to show apparent longitudinal division resulting in both individuals possessing the relatively rare type of nucleus with a distinct karyosome. Whether these forms are destined to play some peculiar part in the life-cycle of the animal is a question which must remain for further work. It is interesting to note that in both these cases the forms possess axostyles, which from their position can hardly have played the important *rôle* in nuclear division which has been ascribed to them by some workers.

It will be evident that *Trichomonas gallinarum* resembles very closely the form described by Prowazek from the lizard.

So far all our attempts at finding any process resembling conjugation have failed. We have found in very large numbers a structure which we must regard as identical with the cyst described by Prowazek, but the cysts by which we find that infection is effected are of a different nature from those described by Prowazek.

To the question of the real nature of this structure we will return in a later part of the paper.

6. TRICHOMONAS EBERTHI.

The features which characterise the second *Trichomonas* form inhabiting the cæca of the fowl have already been mentioned on p. 59, but as in the case of this form we have to deal with a new factor, a rather complicated form change, the equivalent of which has not been met with in *Trichomonas gallinarum*, we have been forced to divide our description under the following heads:

- (1) The typical active *Trichomonas Eberthi*.
- (2) The division of the active form.
- (3) The resting stage.
- (4) The intermediate stages between the resting and the active form.

The Typical Active *Trichomonas Eberthi*.

The body of *Trichomonas Eberthi* is, roughly, of a carrot-shape, measuring, on the average, about 9μ long by $4-6\mu$ broad at its widest point.

Arising from the blunt anterior end there are typically three long free flagella and a membrane flagellum, which runs back in connection with a very broad membrane along the side of the animal, to terminate freely beyond the animal's posterior end (Pl. 12, fig. 33). Near the blunt anterior end a small horn-shaped cytostome is frequently found.

The blepharoplast lies near the anterior end, and consists probably of four darkly staining granules, each of which gives rise to a flagellum. Starting from the blepharoplast, and running back along the base of the membrane to the animal's posterior end, there is a very darkly staining chromatic line.

One of the best-marked differences between *Trichomonas Eberthi* and the previously described *Trichomonas gallinarum* is given, as was stated on p. 59, by the arrangement of the chromatin blocks. These blocks are here much larger and more definite structures, and are arranged in an

irregular double row near the anterior end. They lie over the nucleus in such a way that if the side of the animal down which the line and membrane run be regarded as dorsal, then the blocks are always on the animal's right side. A large and well-developed axostyle is always present. It arises apparently from the blepharoplast, and after a slightly sinuous course ends in a sharp point beyond the animal's posterior end.

The nucleus is a markedly elongated oval structure, and the chromatin is distributed in a coarse meshwork through the substance of the nucleus, showing usually some tendency to become concentrated on the nuclear wall.

Division of the Active Form.

The process of division seems to be always longitudinal. The first indication of division is usually seen in the division of the blepharoplast complex, but as the two new blepharoplasts may remain in close contiguity during the early stages of this process, the first obvious sign of division is given by the outgrowth of the new chromatic line (Pl. 12, figs. 36, 37).

The new chromatic line in *Trichomonas Eberthi* always grows out parallel and in the same sense as the old line, and there is no trace of the chromatic band joining the blepharoplasts which was so characteristic of the dividing forms of *Trichomonas gallinarum*.

The new membrane is formed as an outgrowth down the new line (Pl. 12, fig. 40), but the behaviour of the free flagella during division is a little more difficult to understand.

As has been previously pointed out, the vast majority of active *Trichomonas Eberthi* bear three free anterior flagella. In the division of these forms it seems probable, from a study of stages resembling Pl. 12, fig. 40, that two of the old free anterior flagella pass in the division of the blepharoplast complex to the moiety of the blepharoplast which gives rise to the new chromatic line, the other free

flagellum, with the old membrane flagellum, persisting in its connection with the other moiety of the blepharoplast. The new free flagella, one in the case of the former individual and two in the case of the latter, are probably formed by splitting from the edge of the membrane flagellum (compare Pl. 12, fig. 34). This splitting process, as will be seen later, is also met with in the passage from the resting to the active stage.

We are inclined to regard the relatively rare stages found with more than three free flagella, and with no other sign of division, as examples in which the flagellar apparatus has undergone precocious development in the transition towards the dividing form (Pl. 12, fig. 35).

One of the most characteristic features in the division of *Trichomonas eberthi* is furnished by the behaviour of the chromatic blocks. These at an early stage show a tendency to become arranged in a single line parallel to, and apparently in connection with, the axostyle. During the early stages of division there is a marked tendency of the chromatin in the nucleus to become condensed into a number of masses. In early stages the number of these masses seems to be eight (Pl. 12, fig. 40), but in later stages there is a tendency for them to become paired, giving rise to four double masses (fig. 41). Unfortunately we have no figure showing the actual state of the nucleus at the time of division, but it is probable that the latter is a very modified form of mitosis. We are also unable to supply any really conclusive evidence as to the behaviour of the axostyle during division, but an inspection of a large number of stages leads us to believe that the old axostyle undergoes some process of solution, starting from its anterior end, followed by a re-formation in the case of both individuals along the lines of the chromatic blocks (cf. Pl. 12, figs. 41, 42, 43). This hypothesis may seem at first sight rather improbable to those who regard the axostyle as a relatively permanent skeletal structure, but we have so often watched the development of the axostyle in resting forms of *Trichomastix gallinarum* within a com-

paratively short period that we feel that any objection on this score can carry relatively little weight. On the other hand, we can certainly find no evidence in this form that the axostyle plays the important part in the division of the nucleus which has been assigned to it by some workers on allied forms.

The later stages in division are shown on Pl. 12, figs. 43, 44.

In addition to the active forms of *Trichomonas Eberthi*, relatively rare true resting forms are also met with.

A typical resting form is figured (Pl. 13, fig. 45), in which the nucleus, blocks and well-developed chromatic line are quite normal, but there is no trace of the flagella. These forms are in life characterised by a peculiar movement, during which a conical wave of protoplasm travels slowly at regular intervals down one side of the animal. Every stage can be found between such a resting form and the active *Trichomonas* with its three anterior flagella and well-developed membrane. In the form figured on Pl. 13, fig. 46, the membrane is well developed, and in the presumably later stage, figured in fig. 47, an axostyle is also present.

Part of the changes between such a form as is figured in Pl. 13, fig. 48, and the typical active form have been followed on the living animal. The new free flagella are split successively from the edge of the undulating membrane (cf. Pl. 12, fig. 34).

The new free flagella thus formed at first trail behind the animal, and it is to this fact we believe that the difference in movement so often observed in these *Trichomonads* is due. In the fully developed *Trichomonas* the free flagella strike successively well out in front of the animal, whereas in the trailing form the free flagella drag behind the animal, and the whole movement of translation is probably due to the action of the undulating membrane.

As to the origin of these resting forms, it would be hazardous in the present state of our knowledge to speculate; but we should like in this place to draw attention to another well-marked but little understood series of forms which

possibly play an important part in the life-cycle of the *Trichomonas*. These forms possibly represent some conjugation process, but at present we can offer no decisive evidence upon this point. It is, of course, possible that they are degenerate dividing forms. They may be tersely characterised as forms with a double nuclear apparatus and a double chromatic line, but in which the flagellar apparatus is degenerate, and finally becomes entirely lost. In the later stages of this process a number of forms are met with in which the chromatic lines become twisted into irregular circles.

The nuclei of the specimen shown in Pl. 13, fig. 48, would appear to be undergoing division, possibly preparatory to conjugation, and it is interesting to note that forms of this type with nuclei containing only four chromatic masses have been found, e.g. fig. 49. On the other hand, it is possible that the division of such a form as this would give rise to the resting forms we have previously described.

Against this view, however, it may be noted that on one occasion we had an opportunity of keeping a living form of *Trichomonas Eberthi*, with two membranes and no free flagella, under observation for six hours, during which time there was not only no sign of division, but the animals became more rounded and lost one membrane. It seems very possible that it is to such forms as these that Schaudinn refers on p. 550 in his paper, "Untersuchungen über die Fortpflanzung einiger Rhizopoden" (1903).

"Die in fast jedem Darm vorkommende *Trichomonas intestinalis* verliert nämlich vor der Kopulation ihre Geisseln und ist dann von einer echten Amöbe nicht leicht zu unterscheiden, weil sie auch mit stumpf-lobosen Pseudopodien umherkriecht."

7. *TRICHOMASTIX GALLINARUM*.

The body in the fully developed active form of *Trichomastix gallinarum* is roughly pear-shaped, measuring about 5μ long by 3μ .

From the anterior blunt end four long free flagella take their origin.

Running down one side of the body for a distance of about one third of the animal's length from the anterior end there is a single row of darkly staining chromatic blocks, which seem in most cases to obscure a thin, short, chromatic line. This line is shown in Pl. 13, figs. 52, 53.

The blepharoplast appears to consist of four basal granules, each of which gives rise to one of the four free flagella.

The nucleus is an oval, darkly staining mass lying near the anterior end. Usually the chromatin is scattered more or less irregularly through the substance of the nucleus.

An axostyle is typically present, taking its origin from the blepharoplast, to terminate as a pointed rod at the animal's posterior end. In addition to the elongate active forms, more rounded active forms are also met with in which the axostyle is curved round at its posterior end (Pl. 13, fig. 55). In nearly all these forms, two small darkly staining wedges are found on either side of the axostyle near its posterior end.

We have been able to get a fairly complete series of divisions of the active form of *Trichomastix gallinarum*, though here again we met with a curious time-variable in the behaviour of the various parts of the divided organism to which we have drawn attention above in the account of the division of the other forms.

The division is here, as far as its initial stages are concerned, a longitudinal one. In the earliest stage of division that we figure (Pl. 13, fig. 57) the blepharoplast complex has divided, two of the basal granules with their two attached flagella have remained in the old position near the anterior pole in connection with the old chromatic line. The other two, with their flagella, have moved to a new position in a slightly posterior oblique direction, and have given rise to a new chromatic line. The two new blepharoplasts are still connected by a chromatic band, which is probably analogous with a similar structure described in the division of *Tricho-*

monas gallinarum. The axostyle is apparently unchanged, but the chromatic granules are scattered irregularly through the cytoplasm at the anterior end of the animal. The nucleus has become quite round and the chromatin is now distributed between the nuclear membrane and the karyosome-like body lying in the centre of the nucleus.

We have, unfortunately, no stage showing the behaviour of the nucleus at the moment of division, since in the next stage which we figure (Pl. 13, fig. 58) the nucleus has already divided. The two new blepharoplasts with their attached flagella now lie at opposite sides of the animal's body, which has become more rounded. The band joining the blepharoplasts is already showing signs of disappearance, but that this may persist till a much later stage is shown in figs. 59 and 60. The two new nuclei resemble very closely the nucleus of the preceding fig. 57, since in both cases the chromatin is divided in a characteristic way between the nuclear wall and the internal mass. Here, again, in the case of *Trichomastix gallinarum*, as in the preceding case of *Trichomonas gallinarum*, we have no definite evidence as to the behaviour of the axostyle during division, but we believe that here also we have a process of solution of the old axostyle and the formation of two new ones.

The chromatin blocks in this specimen are still rather irregularly scattered through the cytoplasm. They seem to show some tendency towards their formation into two more or less parallel longitudinal series. In the succeeding stage, shown in fig. 59, the body of the animal is more rounded, and a new short flagellum is growing out from the blepharoplast in each individual. Each individual possesses a short, fine chromatic line and an axostyle, and the chromatin blocks in each case are showing a tendency to resume the arrangement characteristic of the original active individual.

In the next stage (fig. 60) division has proceeded much further as regards the cytoplasm, but the nucleus is in a condition which we must regard as intermediate between the round form with the chromatin divided between the nuclear

membrane and the karyosome, characteristic of the recently divided nucleus figured and the state shown in fig. 59, in which the chromatin is assuming the irregular distribution through an oval nucleus characteristic of the later stages of division and the normal active form.

In the later stages the line between the blepharoplast has disappeared, and the fourth free flagellum has grown out in the case of each individual. The process of division is in these cases complete except for the severance of the cytoplasm. From the occurrence, however, of free individuals with only three free flagella, it seems probable that in some cases the fourth flagellum is only added after the division of the cytoplasm has been effected.

The aflagellate resting forms of *Trichomastix gallinarum* are fairly commonly met with, lying usually in groups of three or four, in preparations from the cæcal wall.

In the typical resting forms, such as are figured on Pl. 14, fig. 64, the body of the animal is quite rounded. There is no trace of flagella, but there is a well-marked blepharoplast. The nucleus has the characteristic appearance already described for the active form, but there is a tendency for the chromatin granules either to become massed in a double row or to fuse, forming a darkly staining band round the periphery. An axostyle may be present or absent.

It is probable that the grouping of these resting forms mentioned above is due to antecedent division.

In fig. 65, Pl. 14, a group of three distinct individuals is drawn, of which A is just dividing, whereas B and C are probably the result of a recent division.

If this hypothesis of division be accepted, it is rather interesting to note that in each of the cases figured (Pl. 14, fig. 65), the division of a presumably non-flagellate form has resulted in the production of one individual in which new flagella are growing out, whilst the other individual still remains in the resting stage. A later stage in the outgrowth of the flagella in a form probably derived from the division of a resting form is seen in fig. 66, Pl. 14.

We have been able to observe the transition between the active elongate form and the resting stage in life on a warm stage.

It will be obvious from the account of the dividing resting form given above that it is possible in these cases for the animals to acquire new free flagella as stiff outgrowths from the blepharoplast, a method which, as we have already seen, is adopted by the dividing individuals in acquiring their two new free flagella. In addition to this process we have met with a number of remarkable swathed forms, which we are inclined to interpret as indicating a process of outgrowth of united flagella along the surface of the cytoplasm. Some of the successive stages, which we are inclined to explain on this hypothesis,¹ are shown in Pl. 14, figs. 67-71.

It is only necessary now to refer to such forms as that figured in Pl. 14, fig. 72. Here, again, as in the analogous forms figured (Pl. 13, figs. 49-52) for *Trichomonas Eberthi*, we possibly have to do with the process of conjugation coupled with a loss of the flagellar apparatus. But here, again, we have no definite evidence to offer in support of this view.

8. EXPLANATION OF PLATES 10-14,

Illustrating the paper by Mr. C. H. Martin and Miss Muriel Robertson upon "The Cæcal Parasites of Fowls," Part I.

[The outlines of all figures were drawn with a camera under Zeiss comp. oc. 12 and 2 mm. apochromat., with long tube at table level. For the study of the detail Zeiss's 1.5 apochromat. with their achromatic condenser Ap. 130 was used.]

PLATE 10.

Figs. 1-10.—*Chilomastix gallinarum*.

Fig. 1.—Active *Chilomastix gallinarum*, showing four free flagella, blepharoplast, nucleus, pharynx and ingested bacteria.

¹ These stages might be regarded as stages in the absorption of the flagella; but we have observed the loss of the flagella in the living form on more than one occasion without any indication of these appearances.

Fig. 2.—Tailed form.

Fig. 3.—Rounded form, showing chromatin masses in the nucleus.

Fig. 4.—Very small form.

Fig. 5.—Early division stage of small *Chilomastix gallinarum*.

Fig. 6.—Later stage of division. Only one of the two flagella is shown in one of the division products.

Fig. 7.—Resting aflagellate form of *Chilomastix gallinarum* prior to encystment.

Fig. 8.—Beginning of the formation of the cyst wall.

Fig. 9.—Late stage in the encystment of *Chilomastix gallinarum*.

Fig. 10.—The cyst of *Chilomastix gallinarum*.

PLATE 11.

Figs. 11-30.—*Trichomonas Gallinarum*.

Fig. 11.—Active *Trichomonas gallinarum*.

Fig. 12.—Elongate active *Trichomonas gallinarum*.

Fig. 13.—*Trichomonas gallinarum* with axostyle.

Fig. 14.—Active form, showing cytostome.

Fig. 15.—First stage of longitudinal division. The blepharoplast has divided; two basal granules with their flagella remain in connection with the old line and membrane flagellum. A new line has grown out from the other two granules.

Fig. 16.—Later stage in longitudinal division. The new blepharoplasts have moved apart, but are connected by a chromatic band.

Fig. 17.—Later stage of longitudinal division.

Fig. 18.—First stage of transverse division.

Fig. 19.—Later stage transverse division.

Fig. 20.—Still later stage of transverse division. This stage shows the arrangement of the chromatin in the nucleus at the time of division.

Fig. 21.—Later stage transverse division.

Fig. 22.—Later stage transverse division.

Fig. 23.—Transverse division.

Fig. 24.—Probably late stage transverse division; the line between the blepharoplasts has disappeared.

Fig. 25.—First stage oblique division.

Fig. 26.—Later stage oblique division.

Fig. 27.—Still later stage oblique division.

Fig. 28.—Late stage division. A third free flagellum is growing out as a short thick rod in one of the daughter individuals.

Fig. 29.—Late stage division, probably longitudinal.

Fig. 30.—Late stage division.

PLATE 12.

Figs. 31, 32.—*Trichomonas gallinarum*.

Fig. 31.—Late stage of the division of *Trichomonas gallinarum*, showing the form with axostyle.

Fig. 32.—Late stage of the division of the form of *Trichomonas gallinarum*, showing the outgrowth of the fourth flagellum in each of the daughter individuals.

Figs. 33-44.—*T. Eberthi*.

Fig. 33.—Active *Trichomonas Eberthi*. Normal form, with three free flagella and membrane flagellum.

Fig. 34.—*Trichomonas Eberthi*. Form with three free flagella and a fourth flagellum just splitting off from the membrane flagellum.

Fig. 35.—*Trichomonas Eberthi*. Form with four free flagella.

Figs. 36-38.—Early stages in division, *Trichomonas Eberthi*.

Fig. 39.—Late stage in division. The chromatin of the nucleus is condensed into eight masses; two new flagella have been formed.

Fig. 40.—Still later stage of division. The chromatin masses have now associated to form four dark masses. The new membrane flagellum is growing out along the new line.

Fig. 41.—Still later stage. The nuclei have divided, though the axostyles are not formed.

Fig. 42.—Late stage division, showing axostyles and divided nuclei.

Figs. 43, 44.—Still later stages in the division of *Trichomonas Eberthi*.

PLATE 13.

Figs. 45-52.—*Trichomonas Eberthi*.

Fig. 45.—A flagellate resting stage of *Trichomonas Eberthi*.

Fig. 46.—*Trichomonas Eberthi*. Form with membrane, but without free flagella.

Fig. 47.—*Trichomonas Eberthi*. Form with membrane and axostyle, but no free flagella.

Fig. 48.—*Trichomonas Eberthi*. Form developing free flagella.

Figs. 49-52.—Doubtful dividing forms *Trichomonas Eberthi*, perhaps really early stages of conjugation.

Figs. 53-63.—*Trichomastix gallinarum*.

Figs. 53, 54.—Active elongate form, *Trichomastix gallinarum*.

Fig. 55.—Active form, *Trichomastix gallinarum*.

Fig. 56.—Active rounded form, showing chromatic line.

Fig. 57.—First stage in division of the active form.

Fig. 58.—Later stage in division.

Fig. 59.—Later stage of division, showing the outgrowth of a new flagellum in each of the daughter individuals.

Fig. 60.—Still later stage.

Fig. 61.—Later stage, in which the chromatic band between the blepharoplasts has disappeared, and the fourth flagellum has been added.

Fig. 62.—Last stage in division.

Fig. 63.—*Trichomastix gallinarum*, with only three free flagella.

PLATE 14.

Figs. 64-72.—*Trichomastix gallinarum*.

Fig. 64.—Resting aflagellate forms of *Trichomastix gallinarum*.

Fig. 65.—Dividing resting forms.

Fig. 66.—*Trichomastix gallinarum*. Possibly further stage in the outgrowth of flagella in an individual, resulting from the division of a resting form.

Fig. 67-71.—Possibly stages in the development of flagella on resting individuals of *Trichomastix gallinarum*.

Fig. 72.—Possibly a stage in conjugation.